Short communication

Intra-periaqueductal grey injection of galanin increases the nociceptive response latency in rats, an effect reversed by naloxone

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Abstract

The nociceptive response latencies were increased significantly after intra-periaqueductal grey (PAG) administration of 1.0 or 3.0 nmol of galanin, but not 0.3 nmol, in rats. The effect of galanin was attenuated by following injection of 5.5 nmol of naloxone into PAG. These results indicate an anti-nociceptive role of galanin, and a possible interaction between galanin and opioid peptides in PAG in rats.

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Keywords: Periaqueductal grey; Nociceptive response latency; Galanin; Opioid peptide; Naloxone

Many studies have shown that the neuropeptide galanin may be involved in the transmission of nociceptive information in the spinal cord [2–4,9,11]. Previous studies in our laboratory also demonstrated that intrathecal administration of galanin resulted in dose-dependent anti-nociceptive effects in the mononeuropathic rats [14]. Nevertheless, the mechanisms of the various actions of galanin, especially in antinociception in the brain, is not yet clearly known. Galanin immunoreactive substance, galanin-immunoreactive fibers and galanin receptors are shown to exist in the rat periaqueductal grey (PAG)[5]. Endogenous opioid peptides are also found in PAG and are demonstrated to contribute significantly to the pain modulation [1,4,8,10]. The present study was performed to investigate the role of intra-PAG injection of galanin, and the possible interaction between galanin and opioid system in rat’s PAG.

Experiments were performed on freely moving male Sprague–Dawley rats (200–300 g; Experimental Animal Center of Beijing Medical University, Beijing, China). All experiments were conducted according to the guideline of the animal ethical committee of Karolinska Institutet and every effort was made to minimize animal suffering. The animals were anaesthetized by intraperitoneal pentobarbital (40 mg/kg) and were mounted on a stereotaxic instrument. A stainless steel guide cannula of 0.8 mm out-diameter was directed to PAG (AP 5.5, L 0.5, H 6.0 mm from the surface of the skull) according to Paxinos and Watson [6], and was fixed to the skull by dental acrylic. All rats were accustomed to the nociceptive tests for 5 days before the surgery. At 2 days after the surgery, intra-PAG injections were performed. On the experimental day, a stainless steel needle with 0.4 mm diameter was directly inserted into the guide cannula, with 1 mm beyond the tip of the latter. One μl of solution was thereafter infused into PAG over 1 min.

The latencies to hindpaw withdrawal during thermal and mechanical stimulation were measured [14–16]. Briefly, the entire ventral surface of the rat’s hindpaw was placed manually on the hot-plate which was maintained at a temperature of 52°C (51.8–52.4°C) [14]. The time to hindpaw withdrawal was measured in seconds (s) to be referred to as the hindpaw withdrawal latency (HWL). The Randall Selitto Test (Ugo Basile, Type 7200, Italy) was used to assess the HWL to mechanical stimulation. A wedge-shaped pusher at a loading rate of 30 g/s was applied to the dorsal surface of the manually handled hindpaw and the latency required to initiate the struggle response was assessed and expressed in seconds. The average values obtained before intra-PAG injection were regarded as the basal HWL. The HWLs recorded during subsequent experiments were expressed as percentage changes of the basal level for each rat (% change of HWL), with a cutoff limit of 15 s to avoid the skin

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Table 1
Effects of galanin administrated into periaqueductal grey on the hindpaw withdrawal latency to thermal and mechanical stimulation in rats

<table>
<thead>
<tr>
<th>Treatments</th>
<th>n</th>
<th>Before (s)</th>
<th>% Change of HWL after intra-periaqueductal grey injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Hot-plate test</td>
<td>10</td>
<td>3.7 ± 0.2</td>
<td>4.3 ± 6.5</td>
</tr>
<tr>
<td>Galanin 0.3 nmol</td>
<td>6</td>
<td>3.6 ± 0.1</td>
<td>0.7 ± 12.5</td>
</tr>
<tr>
<td>Galanin 1 nmol</td>
<td>6</td>
<td>3.9 ± 0.4</td>
<td>25.9 ± 11.4</td>
</tr>
<tr>
<td>Galanin 3 nmol</td>
<td>8</td>
<td>3.4 ± 0.2</td>
<td>12.8 ± 11.0</td>
</tr>
<tr>
<td>Randall–Selitto test</td>
<td>10</td>
<td>5.9 ± 0.2</td>
<td>3.8 ± 3.6</td>
</tr>
<tr>
<td>Galanin 0.3 nmol</td>
<td>6</td>
<td>4.9 ± 0.2</td>
<td>15.5 ± 10.3</td>
</tr>
<tr>
<td>Galanin 1 nmol</td>
<td>6</td>
<td>5.6 ± 0.4</td>
<td>23.0 ± 7.5</td>
</tr>
<tr>
<td>Galanin 3 nmol</td>
<td>8</td>
<td>5.2 ± 0.2</td>
<td>27.2 ± 9.4</td>
</tr>
</tbody>
</table>

Control group: 1 µl of 0.9% saline (n = 10); HWL: hindpaw withdrawal latency.

The data are presented as means ± S.E.M. *P < 0.05, **P < 0.01 and ***P < 0.001 compared with the control group (two-way ANOVA).

Damage. Each rat was tested with both types of stimulation. At the conclusion of the experiments, the location of the tip of the injecting tube was verified and only the results from nociceptive tests with the tips of the injecting tubes placed within PAG area were used for statistical analysis. Data from nociceptive tests were presented as means ± S.E.M. The difference between groups was determined by two-way analysis of variance (ANOVA). *P < 0.05, **P < 0.01 and ***P < 0.001 were considered as significant differences.

Solutions for intra-PAG administration were prepared with sterilized saline, each with a volume of 1 µl: (1) 0.3, 1.0 or 3.0 nmol of galanin (galanin, Bachem, Feinchemikalien, Switzerland), respectively; (2) 5.5 nmol (2 µg) of naloxone (naloxone hydrochloride, Sigma, St. Louis, MO). Control groups were given 1 µl of 0.9% saline.

A total of 30 rats received intra-PAG injection of 0.3 nmol (n = 6), 1.0 nmol (n = 6) or 3.0 nmol (n = 8) of galanin, or 1 µl of 0.9% saline (n = 10) as control group. The results are shown in Table 1. The HWLs to thermal and mechanical stimulation increased significantly after intra-PAG injection of 1.0 nmol (Thermal test: F1/14 = 6.23, P < 0.05; Mechanical test: F1/14 = 35.04, P < 0.001) or 3.0 nmol of galanin (Thermal test: F1/16 = 27.51, P < 0.001; Mechanical test: F1/16 = 42.23, P < 0.001) compared with the control group. In the group receiving intra-PAG injection of 0.3 nmol of galanin, there were no significant changes in HWLs in comparison with the control group (Thermal test: F1/14 = 1.06, P = 0.31; Mechanical test: F1/14 = 2.71, P = 0.11). The effects of intra-PAG administration of 3.0 nmol of galanin reached the peak between 5–10 min, then began to recover from 20 min.

Two groups of rats received intra-PAG injection of 3.0 nmol of galanin, followed 5 min later, by 5.5 nmol of naloxone (n = 10); basal HWLs were 4.8 ± 0.3 s for of thermal tests and 6.1 ± 0.2 s for mechanical tests) or 1 µl of 0.9% saline (n = 10; basal HWLs were 4.6 ± 0.3 s for of thermal tests and 6.7 ± 0.4 s for mechanical tests). In our experiments, 5 min after intra-PAG injection of 5.5 nmol of naloxone, the increased HWL was attenuated partially in both tests (Fig. 1A, Thermal test: F1/18 = 43.67, P < 0.001; Fig. 1B, Mechanical test: F1/18 = 34.39, P < 0.001) in comparison with the control group, in which the HWLs to both noxious stimulation increased for about 30 min, as shown in Fig. 1. Another group of rats (n = 5), received intra-PAG administration of 1 µl of saline, fol-
lowed 5 min later by 5.5 nmol of naloxone, showed no significant changes of HWLs to either stimulation.

Yaksh et al. [13] reported that the antinociceptive effects induced by intra-PAG injection of morphine were reversed by following intra-PAG administration of naloxone. The results of the present study showed clearly that the nociceptive response latencies were increased significantly after intra-PAG administration of 1.0 or 3.0 nmol of galanin, but not 0.3 nmol in rats, indicating that in PAG galanin may be involved in the endogenous antinociceptive system. Studies suggested that in the spinal cord galanin and opioids played synergic effects in the transmission of presumed antinociceptive information [7,12]. In the present study, we demonstrated that the increase in HWLs induced by intra-PAG administration of galanin could be attenuated by the opioid antagonist naloxone, indicating a possible interaction of antinociception between galanin and opioid in PAG.

Acknowledgements

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References